

I claim:

1. A method for diagnosing cancer or cancer-related conditions from tissue samples, comprising:

(a) obtaining a tissue sample from a test tissue or region to be diagnosed;
(b) performing a methylation assay of the tissue sample, wherein the methylation assay determines the methylation state of genomic CpG sequences, wherein the genomic CpG sequences are located within at least one gene sequence selected from the group consisting of *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, *TYMS* and *MTHFR*, and combinations thereof; and
(c) making a diagnostic or prognostic prediction of the cancer based, at least in part, upon the methylation state of the genomic CpG sequences.

2. The method of claim 1, wherein the genomic CpG sequences located within at least one gene sequence selected from the group consisting of *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2* and *TYMS*, correspond to genomic CpG sequences of CpG islands.

3. The method of claim 1, wherein the *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, *TYMS* and *MTHFR* gene sequences are those defined by the specific oligonucleotide primers and probes corresponding to SEQ ID Nos:1-60, 64 and 65, as listed in TABLE II, or portions thereof.

4. The method of claim 2 wherein the CpG islands are located within the promoter regions of one or more of the *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2* and *TYMS* genes.

5. The method of claim 2, wherein the *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, and *TYMS* gene sequences correspond to any CpG island sequences associated with the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:1-54, 58-60, 64 and 65, as listed in TABLE II, or portions thereof, and wherein the associated CpG island sequences are those contiguous sequences of genomic DNA that encompass at least one nucleotide of the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:1-54, 58-60, 64 and 65, and satisfy the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5.

6. The method of claim 1, wherein the genomic CpG sequences are located within at

least one gene sequence selected from the group consisting of *APC*, *CDKN2A*, *MYODI*, *CALCA*, *ESRI*, *MGMT* and *TIMP3*, and combinations thereof.

7. The method of claim 6, wherein the genomic CpG sequences located within at least one gene sequence selected from the group consisting of *APC*, *CDKN2A*, *MYODI*, *CALCA*, *ESRI*, *MGMT* and *TIMP3*, correspond to genomic CpG sequences of CpG islands.

8. The method of claim 6, wherein the *APC*, *CDKN2A*, *MYODI*, *CALCA*, *ESRI*, *MGMT* and *TIMP3* gene sequences are those defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:19-21, SEQ ID NOs:1-3, SEQ ID NOs:7-9, SEQ ID NOs:10-12, SEQ ID NOs:4-6, SEQ ID NOs:16-18 and SEQ ID NOs:13-15, respectively, as listed in TABLE II.

9. The method of claim 7 wherein the CpG islands are located within the promoter regions of one or more of the *APC*, *CDKN2A*, *MYODI*, *CALCA*, *ESRI*, *MGMT* and *TIMP3* genes.

10. The method of claim 7 wherein the *APC*, *CDKN2A*, *MYODI*, *CALCA*, *ESRI*, *MGMT* and *TIMP3* gene sequences correspond to any CpG island sequences associated with the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:19-21, SEQ ID NOs:1-3, SEQ ID NOs:7-9, SEQ ID NOs:10-12, SEQ ID NOs:4-6, SEQ ID NOs:16-18 and SEQ ID NOs:13-15, respectively, as listed in TABLE II, or portions thereof, and wherein the associated CpG island sequences are those contiguous sequences of genomic DNA that encompass at least one nucleotide of the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:19-21, SEQ ID NOs:1-3, SEQ ID NOs:7-9, SEQ ID NOs:10-12, SEQ ID NOs:4-6, SEQ ID NOs:16-18 and SEQ ID NOs:13-15, and satisfy the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5.

11. The method of claim 1, wherein the cancer or cancer-related condition is selected from the group consisting of gastrointestinal or esophageal adenocarcinoma, gastrointestinal or esophageal dysplasia, gastrointestinal or esophageal metaplasia, Barrett's intestinal tissue, pre-cancerous conditions in normal esophageal squamous mucosa, and combinations thereof.

12. The method of claim 11, wherein the cancer is esophageal adenocarcinoma, and wherein making a diagnostic or prognostic prediction of the cancer, based upon the methylation state of the genomic CpG sequences provides for classification of the adenocarcinoma by grade or stage.

13. The method of claim 6, wherein the cancer or cancer-related condition is selected from the group consisting of gastrointestinal or esophageal adenocarcinoma, gastrointestinal or esophageal dysplasia, gastrointestinal or esophageal metaplasia, Barrett's intestinal tissue, pre-cancerous conditions in normal esophageal squamous mucosa, and combinations thereof.

14. The method of claim 13, wherein the cancer is esophageal adenocarcinoma, and

wherein making a diagnostic or prognostic prediction of the cancer, based upon the methylation state of the genomic CpG sequences provides for classification of the adenocarcinoma by grade or stage.

15. The method of claim 1, wherein the methylation assay used to determine the methylation state of genomic CpG sequences is selected from the group consisting of MethylLight™, MS-SNuPE, MSP, COBRA, MCA, and DMH, and combinations thereof.

16. The method of claim 6, wherein the methylation assay used to determine the methylation state of genomic CpG sequences is selected from the group consisting of MethylLight™, MS-SNuPE, MSP, COBRA, MCA and DMH, and combinations thereof.

17. The method of claim 1, wherein the methylation assay used to determine the methylation state of genomic CpG sequences is based, at least in part, on an array or microarray comprising CpG-containing sequences located within at least one gene sequence selected from the group consisting of *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, *TYMS* and *MTHFR*.

18. The method of claim 17, wherein the *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, and *TYMS* gene sequences correspond to any CpG island sequences associated with the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:1-54, 58-60, 64 and 65, as listed in TABLE II, or portions thereof, and wherein the associated CpG island sequences are those contiguous sequences of genomic DNA that encompass at least one nucleotide of the sequences defined by the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:1-54, 58-60, 64 and 65, and satisfy the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5.

19. The method of claim 17, wherein the *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*, *TGFBR2*, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, *TYMS* and *MTHFR* gene sequences are those defined by, or correspond to the specific oligonucleotide primers and probes corresponding to SEQ ID NOs:1-60, 64 and 65, as listed in TABLE II, or portions thereof.

20. The method of claim 1 wherein the methylation state of genomic CpG sequences that is determined is that of hypermethylation, hypomethylation or normal methylation.

21. A kit useful for diagnosis or prognosis of cancer or cancer-related conditions, comprising a carrier means containing one or more containers comprising:

(a) a container containing a probe or primer which hybridizes to any region of a sequence located within at least one gene sequence selected from the group consisting of *APC*, *ARF*, *CALCA*, *CDH1*, *CDKN2A*, *CDKN2B*, *ESR1*, *GSTP1*, *HIC1*, *MGMT*, *MLH1*, *MYOD1*, *RB1*,

TGFBR2, *THBS1*, *TIMP3*, *CTNNB1*, *PTGS2*, *TYMS* and *MTHFR*; and

(b) additional standard methylation assay reagents required to affect detection of methylated CpG-containing nucleic acid based, at least in part, on the probe or primer.

5 22. The kit of claim 21, wherein the additional standard methylation assay reagents are standard reagents for performing a methylation assay from the group consisting of MethyLight™, MS-SNuPE, MSP, COBRA, MCA and DMH, and combinations thereof.

23. The kit of claim 21, wherein the probe or primer comprises at least about 12 to 15 nucleotides of a sequence selected from the group consisting of SEQ ID NOs:1-60, 64 and 65, as listed in TABLE II.

10 24. A kit useful for diagnosis or prognosis of cancer or cancer-related conditions, comprising a carrier means containing one or more containers comprising:

(a) an array or micorarray comprising sequences of at least about 12 to 15 nucleotides of a sequence selected from the group consisting of SEQ ID NOs:1-60, 64, 65, and any sequence located within a CpG island sequence associated with SEQ ID NOs:1-54, 58-60, 64 and 65.